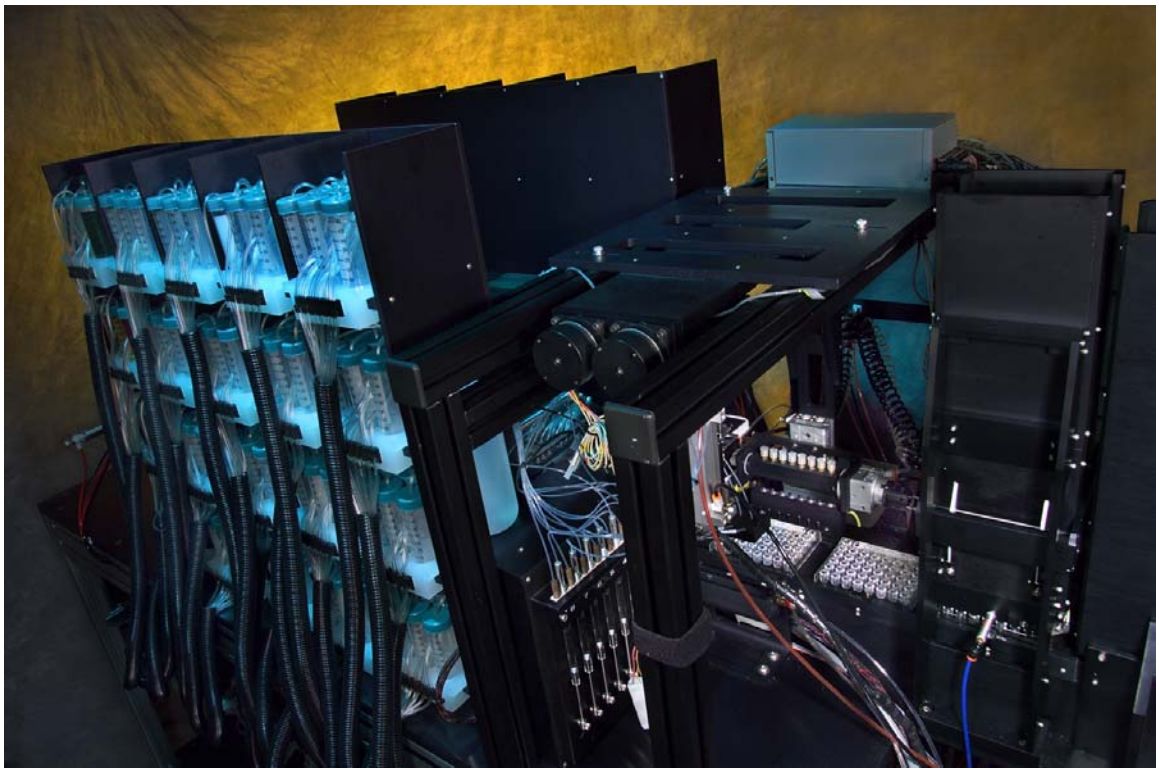


High-Throughput Nano-droplet Protein Crystallization Robot

Begun in the spring of 1998, the BioInstrumentation Group undertook to increase the throughput of protein crystallization trials by an order of magnitude while reducing protein usage per trial by 90%. In order to achieve this goal, two automated robotic systems were designed and constructed with initial testing of the completed system in the spring of 1999. Joining a combinatorial approach with parallel processes, protein samples are screened against a coarse array of up to 480 unique solutions at a rate of eight seconds/trial. This compares with a rate of one to two minutes/trial either by commercial robots or manual methods. Along with the increased throughput, protein usage was reduced up to 50-fold where only 20 nanoliters of protein/trial were needed to grow crystals compared to current best practices using one microliter of protein. Based on sub-optimal growth conditions identified by hits from the coarse screen, a second system narrows in on the optimal conditions by synthesizing a new two-dimensional array of solutions out of 72 stock chemicals and new crystallization trials begin. By automating crystal growth trials, effort previously spent on manual trial preparation is freed to work on other aspects of proteomic research.

Up to 35 plates (either empty or filled with mother liquid) are placed into the input stacker (Figure 1). As each plate advances, a barcode reader captures the unique barcode identifier and the database is queried for instructions (Figure 2). Once the lid is removed,

the plate advances and grease is applied to the rims of all 48 wells (Figure 3). Based on the initial user input, the plate will be filled from one of the 10 dispensing stations; each station having 48 unique mother liquids (varying in reagents, pH, salt concentrations, detergents, etc.) (Figure 4). Once in position at the trial setup station, a variety of tasks commence. Mother liquid is aspirated from each row with an 8-channel dispenser and a single drop (from 20 nanoliters up) is dispensed onto thin glass coverslips (held in place with a vacuum chuck). A single channel, non-contact dispenser then applies a drop of protein (selected from a chilled block containing up to eleven vials of protein) on top of the mother liquid drop at a volume selected by the user (Figures 5 and 6). The glass coverslips are then sealed on the row from which the mother liquid was withdrawn for that trial. This process is repeated five more times for each plate, thus setting up 48 unique crystallization trials in the space of a few minutes. A lid is than applied, the plate moves into the output stack , and once the run is complete, the entire stack is placed in a temperature controlled room where the crystallization process proceeds. Depending on the size of the drops, the mother liquids and proteins used, crystals may form anywhere from hours to weeks later (Figure 7).

This technology and process has been awarded Patent #6,296,673 and was licensed to the start-up firm Syrxx in 2000. This past fall, LBNL was honored to receive a 2002 R&D 100 Award for technological innovation and significance displayed by the automated protein crystallization system.

Figure 1.

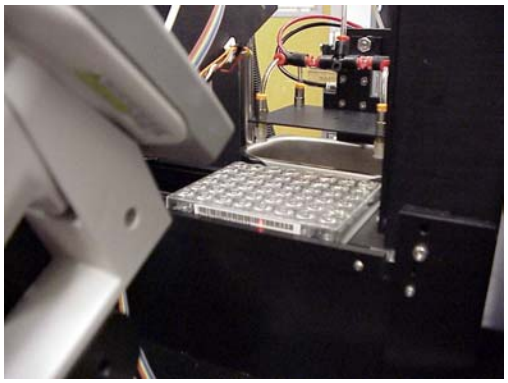


Figure 2.

Figure 3.

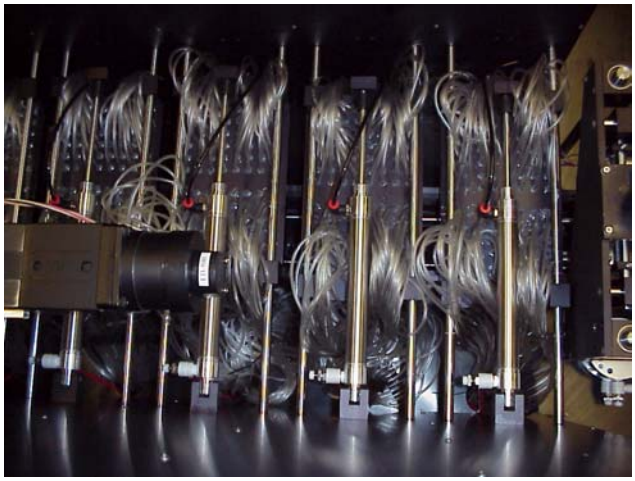
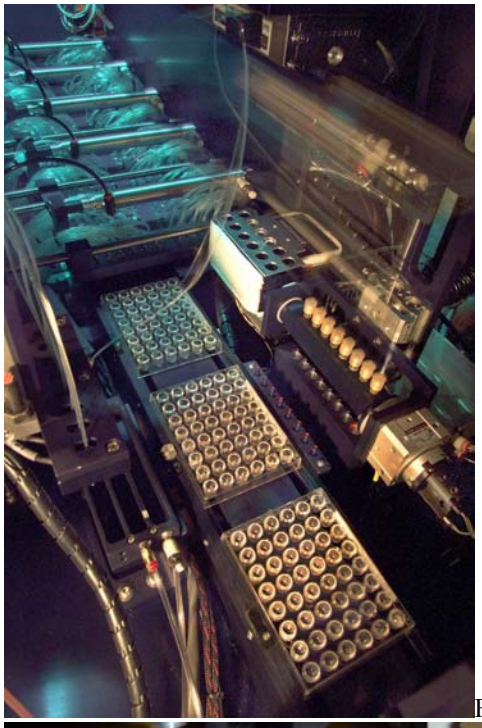


Figure 4.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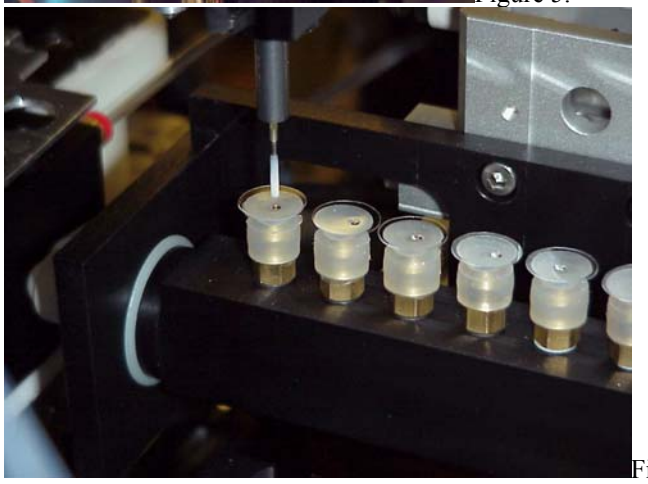
Figure 6.

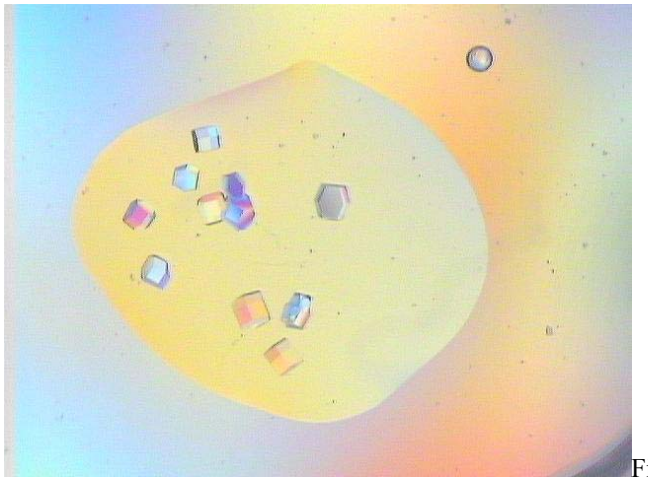
Figure 7.